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(73) Proprietor: **COOPER LABORATORIES**
455 East Middlefield Road
Mountain View California 94039(US)

Proprietor: **Crystal, Ronald G.**
3746 Cumberland Street, N.W.
Washington, D.C. 20016(US)

(72) Inventor: **Roosdorp, Nicolaas J.**
808 Byrd Lane
Foster City, CA 94404(US)
Inventor: **Crystal, Ronald G.**
3746 Cumberland Street, N.W.
Washington, D.C. 20016(US)

(74) Representative: **Harrison, David Christopher et al**
MEWBURN ELLIS & CO 2/3 Cursitor Street
London EC4A 1BQ(GB)

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Description

The subject field is the use of aerosolization for administration of proteinaceous drugs to the lungs.

In the treatment of many diseases, there is a continuing problem of administration. Many therapeutic agents cannot be administered orally, since they will be subject to degradation or other processing in the digestive tract, resulting in loss of efficacy. Proteins are particularly sensitive to the action of proteases resulting in their hydrolysis and fragmentation. Another mode of administration which may be used is parenteral where the therapeutic agent may be injected at a variety of sites, such as subcutaneously, intraarterially, intravenously, intraperitoneally, or the like. For the most part, unless the diseased state is at the site of injection and in many cases even where the diseased state is at the site of injection, the drug is rapidly degraded and/or distributed throughout the vascular system. Thus, the host is systemically affected by the drug, where the major proportion of the drug merely serves to maintain a minimum level in the bloodstream, so that the diseased state is treated with a therapeutic dosage. Where drugs have side effects other than the desired result, and most drugs do, the result is substantial interference with normal bodily functions.

An alternative route which has been considered in some instances is administration intranasally which again results in systemic deposition of the drug, aerosolization. Where the concern is a diseased state of the lungs, the drug may be directly delivered to the diseased tissue by aerosolization and subsequent inhalation of the drug. However, even where one directs the drug to the lungs initially, there are substantial uncertainties about the efficacy in treating the lungs. The half-life of the drug in the lungs may be relatively short due to absorption into the vascular system. In addition, those drugs which are sensitive to enzymatic degradation or other processing, will be subject to modification and loss of efficacy. There is also the problem of the effect of aerosolization on the drug, where the drug may be degraded by the nebulizing action of the nebulizer or inactivated by oxidation There is also the uncertainty of the distribution of the drug in the lungs, as well as the ability to maintain an effective dosage for an extended period, without detrimental effect to the lungs or other organs of the host.

Relevant Literature

U.S. Patent No. 4,599,311 describes the preparation of α_1-antitrypsin by recombinant techniques.

A number of studies have been directed to aerosol inhalation to determine the effect and fate of antigenic proteins which were included in the aerosol. See for example, Brailey et al., J. Immunol. (1978) 121:926-929; Brailey et al., J. Clin. Invest. (1979) 63:1103-1109; and Dawson et al., Chest (1979) 75:(2 suppl.) 276-278 who studied the effects and fate of human serum albumin and ovalbumin. Willoughby and Willoughby, J. Immunol. - (1977) 119:2137-2146; Willoughby et al., Lab Invest. (1979) 40:399-414; and Shenker et al., J. Immunol. (1980) 124:1763-1772 studied the effect and fates, either independently or combined, of the antigens Concanavalin A and bovine serum albumin. Other references concerned with antigens and aerosols include Karol. Amm. Ind. Hyg. Assoc. J. (1979) 40:283-290; Hogg et al., Fed. Proc. (1979) 38:197-201; and Higginbothan et al., Food Chem. Toxicol. (1983) 21:815-823.

The preparation of protein containing aerosols has been described by Przyborowsky, Eur. J. Nucl. Med. (1982) 7:71-72, as well as the above identified references.

The administation of insulin in an aerosol for th treatment of diabetes is described by Wigley et al., Diabetes (1971) 20:552-556 and Yoshida et al., J. Pharm. Sci. (1979) 670-671.

According to the invention, an aerosol formulation for use in the prophylactic or therapeutic treatment of diseased states of the lung contains a serine protease inhibitor protein, preferably in a quantity of 0.1 to 15% weight percent of the aerosol agent. The invention is exemplified by the use of recombinant α_1-antitrypsin to inhibit elastase, a proteolytic enzyme affecting lung tissue and implicated as a major cause of emphysema.

In specific embodiments of the invention methods and compositions are provided for treating diseased states of the lung where the therapeutic agent is a protein, particularly a high molecular weight protein. Aerosol formulations are prepared of the protein to provide a physiologically effective dosage of the protein in the lungs to provide a prophylactic or therapeutic effective amount. It is found that the drugs are retained in the lung epithelial lining fluid, so as to maintain an effective concentration in the lung in contact with lung tissue for extended periods of time.

The subject invention is of particular interest in that it employs proteinaceous drugs which serve as serine protease inhibitors, particularly the use of α_1-antitrypsin as an inhibitor of elastase. The α_1-antitrypsin may be used in the treatment of emphysema to prevent proteolyte attack on native lung tissue. The drugs which are employed may be naturally occurring, that is, isolated from natural sources, may be prepared by recombinant or synthetic techniques, may be mutants of naturally occuring drugs or non-naturally occurring drugs or

combinations thereof.

The subject invention can be used with a variety of lung associated diseases where protein compositions may find application. The proteins for the most part will be not less than 5,000 molecular weight (5kD), generally at least 15kD molecular weight, usually at least about 20kD molecular weight, more usually at least about 30kD molecular weight, frequently exceeding 50kD molecular weight, and may be 600kD or more molecular weight, usually not exceeding about 500kD molecular weight. Diseases other than those directly related to infection which may be treated include asthma, adult or infant respiratory distress syndrome, emphysema, lung cancer, etc.

The aerosol formulation may be varied widely, depending upon the nature of the therapeutic agent, whether additional agents will be included, the manner and area in which it will be released in the lungs, or the like.

The amount of protein which is employed will usually vary from 0.1 to 15, more usually 0.5 to 10, weight percent of the aerosol agent. Other components which may be included include excipients, which are water soluble and may also serve to enhance absorption. These additives include lactose. In addition, physiologically acceptable surfactants may be employed, particularly glycerides, more particularly diglycerides, where one of the carboxylic acids is of from 2 to 4 carbon atoms, and the other will be of from 12 to 20 carbon atoms, more usually of from 16 to 18 carbon atoms, either saturated or unsaturated.

The excipient may vary from 0 to 80 weight percent of the formulation, while the surfactant may vary from 10 to 50 weight percent of the formulation.

Various physiologically acceptable inert gases may be employed as the aerosolizing agent or a nebulizer may be used to form the desired size aerosol particles. Where an inert gas is employed, such as polyhaloalkanes, e.g.. dichlorodifluoromethane, dichlorotetrafluoroethane, etc., these will normally be present in 0.5 to 5 weight percent.

For α_1-antitrypsin, the amount employed will vary depending upon a number of factors, including the size of the particle, frequency of administration, nature of the disease, whether the treatment is for therapeutic or prophylactic purposes, etc. Usually the dosage will vary from 1μg to 10mg/kg of host. The diameter of the particles will generally range from 0.5 to 5μm, preferably from 1 to 3μm. The period of treatment will vary widely, depending upon the therapeutic dosage, the concentration of the drug, the rate of administration, and the like. Generally, the period of administration will range from 2 sec to 30 min, more usually from 3 sec to 7 sec with metered dose inhalers and 10 to 20 min for nebulizers. A single administation or repeated administrations may be required. Thus, the aerosol may be administered one or more times at intervals from 2 to 24 hours.

The following examples are offered by way of illustration of the invention.

EXPERIMENTAL

In order to demonstrate that recombinant α_1-antitrypsin (rAAT) could be delivered to the lower respiratory tract and traverse the lung tissue, the following experiment was performed.

Surgical Procedure

An animal model was employed as described by Staub et al., J. Surgical Res. (1975) 19:315-320. By utilizing a chronic lung lymph fistula, nearly pure lung lymph may be isolated. Sheep are anesthetized with 0.5g sodium pentothal, intubated, and ventilated with room air and 0.5-1% halothane. Catheters are installed in the carotid artery and jugular vein and a right thoracotomy is performed to install a chronic catheter in the afferent lymph duct from the caudal mediastinal node. The tail of the node is ligated and the systemic lymphatics along the diaphragm are cut to minimize the contribution of systemic lymph to the lymph that is collected. After closing the chest, the sheep are allowed to recover for 2-3 days before experimentation.

On the day of the test, the test sheep are anesthetized with sodium pentothal, intubated, and ventilated with room air and halothane as described previously. The appropriate amount of α_1-antitrypsin as a 25mg/ml rAAT in PBS (phosphate-buffered saline) is put into a Heyer Model USC77 ultrasonic nebulizer and the nebulizer is inserted in the inspired line from the ventilator to the sheep's endotracheal tube. With the sheep under the gamma camera and the ventilator set at a tidal volume of 19ml/kg body weight the nebulizer is turned on to allow generation of the aerosol. After approximately 2 minutes, the nebulizer is turned off and removed from the circuit. AAt in normal PBS.

Once every 5 experiments, the size of the aerosol particle entering the endotracheal tube is measured by drawing a sample of the inspired air through a cascade impactor during the deposition period. The measurements show that the aerosol produced has a mass mean aerodynamic diameter of approximately 1.2μm and a geometric standard deviation of 1.6.

Purified recombinant α_1-antitrypsin (prepared essentially as described in U.S. Patent No. 4,599,311) was administered intravenously or by

aerosol to sheep (n = 11) as previously described and the concentration of the α_1-antitrypsin was measured in plasma, lung epithelial lining fluid (ELF) and lung lymph. Using a dose of 60mg/kg, intravenous infusion resulted in lung ELF levels of 400 ±100nM after 2h. In contrast, using a nebulization system that generated greater than 95% of particles of less than about 5μm and 34 ±2% less than 2μm, a dose of only 2.5mg/kg of aerosolized α_1-antitrypsin resulted in the same ELF levels at 2h ($p>0.1$). The aerosolized α_1-antitrypsin appeared in lung lymph in a time dependent manner (1h 2 ±1nM, 2h 13 ±6 nM, 3h 27 ±17nM, 4h 117 ±30nM), driven by a concentration gradient in ELF (2h 400 ±50nM).

The above results demonstrate that the α_1-antitrypsin can be aerosolized into sufficient sized particles to provide therapeutic dosage levels of proteins, to access the alveolar spaces and in vivo reach the epithelial surface and interstitium of the lower respiratory tract. Thus, the aerosolized administered α_1-antitrypsin can augment the anti-elastase defenses related to protection against inflammatory action and prevent attack of normal tissue.

The success demonstrated above is paradigmatic of the fact that a high molecular weight protein that is oxidation sensitive can be aerosolized and retain its activity. The protein may be delivered to the lower respiratory tract in active form. Aerosolization provides for transport of large proteins through the lung epithelial lining to the lung interstitium and final delivery to the blood for clearance. The aerosolization is able to provide for protection of the molecule from degradation by tissue proteases or cellular uptake. The aerosol administered protein also appears to be protected from rapid removal and/or inactivation for extended periods of time to allow for long-term effectiveness, so as to reduce the required number of administrations.

Claims

Claims for the following Contracting States : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. An aerosol formulation for prophylactic or therapeutic treatment of the lungs comprising a protein which is a serine protease inhibitor, in a quantity effective for said treatment.

2. An aerosol formulation according to claim 1 wherein the protein is present in a quantity of 0.1 to 15 weight percent of the aerosol agent.

3. An aerosol formulation according to claim 2 wherein said serine protease inhibitor is present in from 0.5 to 10 weight percent of the aerosol agent.

4. An aerosol formulation according to any of the preceding claims wherein said serine protease inhibitor is α_1-antitrypsin.

5. An aerosol formulation according to any of the preceding claims wherein said serine protease inhibitor is of at least 30 kD.

6. An aerosol formulation according to any of the preceding claims wherein said serine protease inhibitor is produced by recombinant techniques.

7. An aerosol formulation according to any of the preceding claims wherein said serine protease inhibitor is produced in yeast.

8. An aerosol formulation according to any of the preceding claims wherein said serine protease inhibitor is of at least 50 kD.

9. An aerosol formulation according to any of the preceding claims wherein said serine protease inhibitor is in the form of particles predominantly in the range of 0.5 to 5 micrometers in diameter.

10. An aerosol formulation according to any of the preceding claims wherein said particles are predominantly in the range of 1 to 3 micrometers in diameter.

Claims for the following Contracting States : ES, GR

1. A method of making an aerosol formulation used for prophylactic or therapeutic treatment of the lungs comprising incorporating in said formulation a protein which is a serine protease inhibitor in a quantity effective for said treatment.

2. A method according to claim 1, wherein said protein is incorporated in from 0.1 to 15 weight percent, based on the aerosol agent.

3. A method according to claim 2 wherein said protein is incorporated in from 0.5 to 10 weight percent.

4. A method according to any of claims 1, 2 or 3 wherein said protein agent is α_1-trypsin.

5. A method according to any one of the preceding claims, wherein said protein is of at least 30 kD.

6. A method according to any one of the preceding claims, wherein said protein agent is produced by recombinant techniques.

7. A method according to claim 6, wherein said protein agent is produced in yeast.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Aerosolformulierung zur prophylaktischen oder therapeutischen Behandlung der Lungen, die ein Protein, das ein Serinproteaseinhibitor ist, in einer Menge umfaßt, die für die genannte Behandlung wirksam ist.

2. Aerosolformulierung nach Anspruch 1, worin das Protein in einer Menge von 0,1 bis 15 Gew.-% des Aerosolagens vorliegt.

3. Aerosolformulierung nach Anspruch 2, worin der genannte Serinproteaseinhibitor in einer Menge von von 0,5 bis 10 Gew.-% des Aerosolagens vorliegt.

4. Aerosolformulierung nach einem der vorhergehenden Ansprüche, worin der genannte Serinproteaseinhibitor α_1-Antitrypsin ist.

5. Aerosolformulierung nach einem der vorhergehenden Ansprüche, worin der genannte Serinproteaseinhibitor zumindest 30 kD aufweist.

6. Aerosolformulierung nach einem der vorhergehenden Ansprüche, worin der genannte Serinproteaseinhibitor durch rekombinante Techniken hergestellt ist.

7. Aerosolformulierung nach einem der vorhergehenden Ansprüche, worin der genannte Serinproteaseinhibitor in Hefe hergestellt ist.

8. Aerosolformulierung nach einem der vorhergehenden Ansprüche, worin der genannte Serinproteaseinhibitor zumindest 50 kD aufweist.

9. Aerosolformulierung nach einem der vorhergehenden Ansprüche, worin der genannte Serinproteaseinhibitor in der Form von Teilchen mit einem Durchmesser vorwiegend im Bereich von 0,5 bis 5 µm vorliegt.

10. Aerosolformulierung nach einem der vorhergehenden Ansprüche, worin die genannten Teilchen einen Durchmesser vorwiegend im Bereich von 1 bis 3 µm aufweisen.

Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Verfahren zur Herstellung einer Aerosolformulierung, die zur prophylaktischen oder therapeutischen Behandlung der Lungen eingesetzt wird, umfassend das Einbinden eines Proteins in die genannte Formulierung, das ein Serinproteaseinhibitor ist, in einer Menge, die für die genannte Behandlung wirksam ist.

2. Verfahren nach Anspruch 1, worin das genannte Protein in einer Menge von 0,1 bis 15 Gew.-%, bezogen auf das Aerosolagens, vorliegt.

3. Verfahren nach Anspruch 2, worin das genannte Protein in einer Menge von von 0,5 bis 10 Gew.-% eingebunden ist.

4. Verfahren nach einem der Ansprüche 1, 2 oder 3, worin das genannte Proteinagens α_1-Antitrypsin ist.

5. Verfahren nach einem der vorhergehenden Ansprüche, worin das genannte Protein zumindest 30 kD aufweist.

6. Verfahren nach einem der vorhergehenden Ansprüche, worin das genannte Proteinagens durch rekombinante Techniken hergestellt wird.

7. Verfahren nach Anspruch 6, worin das genannte Proteinagens in Hefe hergestellt wird.

Revendications

Revendications pour les Etats contractants sulvants : AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Formule en aérosol pour le traitement prophylactique ou thérapeutique des poumons, comprenant une protéine qui est un inhibiteur de la protéase de la sérine, en une quantité efficace pour ledit traitement.

2. Formule en aérosol selon la revendication 1, où la protéine est présente en une quantité de 0,1 à 15 pour cent en poids de l'agent aérosol.

3. Formule en aérosol selon la revendication 2, où ledit inhibiteur de la protéase de la sérine est présent à raison de 0,5 à 10 pour cent en poids de l'agent aérosol.

4. Formule en aérosol selon l'une quelconque des revendications précédentes, où ledit inhibiteur de la protéase de la sérine est l'α_1-anti-

trypsine.

5. Formule en aérosol selon l'une quelconque des revendications précédentes, où ledit inhibiteur de la protéase de la sérine est d'au moins 30 kD.

6. Formule en aérosol selon l'une quelconque des revendications précédentes, où ledit inhibiteur de la protéase de la sérine est produit par des techniques recombinantes.

7. Formule en aérosol selon l'une quelconque des revendications précédentes, où ledit inhibiteur de la protéase de la sérine est produit dans une levure.

8. Formule en aérosol selon l'une quelconque des revendications précédentes, où ledit inhibiteur de la protéase de la sérine est d'au moins 50 kD.

9. Formule en aérosol selon l'une quelconque des revendications précédentes, où ledit inhibiteur de la protéase de la sérine a la forme de particules qui sont de façon prédominante comprises entre 0,5 et 5 micromètres de diamètre.

10. Formule en aérosol selon l'une quelconque des revendications précédentes, où lesdites particules sont de manière prédominante comprises entre 1 et 3 micromètres de diamètre.

Revendications pour les Etats contractants suivants : ES, GR

1. Méthode de production d'une formule en aérosol utilisée pour le traitement prophylactique ou thérapeutique des poumons, comprenant l'incorporation, dans ladite formule, d'une protéine qui est un inhibiteur de la protéase de la sérine en une quantité efficace pour ledit traitement.

2. Méthode selon la revendication 1, où ladite protéine est incorporée à raison de 0,1 à 15 pour cent en poids en se basant sur l'agent aérosol.

3. Méthode selon la revendication 2, où ladite protéine est incorporée à raison de 0,5 à 10 pour cent en poids.

4. Méthode selon l'une quelconque des revendications 1, 2 ou 3, où ledit agent protéique est l'α_1-trypsine.

5. Méthode selon l'une quelconque des revendications précédentes, où ladite protéine est d'au moins 30 kD.

6. Méthode selon l'une quelconque des revendications précédentes, où ledit agent protéique est produit par techniques recombinantes.

7. Méthode selon la revendication 6, où ledit agent protéique est produit dans une levure.